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What's New In General Surgery

The Development of New Immunotherapies for the Treatment of Cancer Using Interleukin-2

A Review

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Recent increases in knowledge of cellular immunology, combined with developments in biotechnology, have provided new opportunities for the development of immunotherapies for the treatment of cancer in humans. One approach to therapy is that of adoptive immunotherapy, that is, the transfer to the tumor bearing host of lymphoid cells with antitumor reactivity that can mediate antitumor responses. Several lymphocyte subpopulations have now been identified that may be suitable for use in adoptive immunotherapy. Resting lymphocytes incubated in interleukin-2 (IL-2) give rise to lymphokine activated killer (LAK) cells that can lyse malignant cells, but not normal cells. Clinical studies in patients with advanced cancer have revealed that treatment with high dose IL-2 alone or in combination with LAK cells can mediate the complete or partial regression of cancer in selected patients. Other approaches are currently undergoing investigation, including the adoptive transfer of tumor infiltrating lymphocytes, which, in animal models, have antitumor reactivity 50–100 times more potent than do LAK cells. Other new approaches to immunotherapy include the use of combinations of lymphokines, such as the use of tumor necrosis factor or alpha interferon in conjunction with IL-2. The availability of recombinant lymphokines that provide large amounts of biologically active materials can hopefully lead to the development of effective new therapies for cancer in humans.

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SURGERY IS THE TREATMENT most effective in curing patients with invasive cancer. Of the 930,000 Americans who were diagnosed with invasive cancer in 1986, approximately one half will be cured using currently available treatment. Approximately two thirds of those cured will be cured by surgical resection alone, and others by the combination of surgery and other treatments. Yet even with the best available treatment, approximately 50% of all newly diagnosed cancer patients will die of metastatic cancer. Intensive efforts have therefore been devoted to developing new modalities for treating cancer, and the development of biologic therapies has been foremost in these efforts.

In the hope that a general increase in host immune reactivity will lead to a concomitant increase in the immune response against growing tumors, most attempts to develop immunotherapies for the treatment of cancer have involved nonspecifically stimulating the body's immune reactivity with general immunostimulants, such as BCG or C. parvum. Though this approach has

TABLE 1. *Generation of LAK Cells in Mice and Humans*

Mouse			Human		
Target	Fresh Splenocytes	Splenocytes Cultured in IL-2	Target	Fresh PBL	PBL Cultured in IL-2
		(% lysis)*			
MCA 102 sarcoma	1 ± 1	53 ± 2	Autologous sarcoma	−4 ± 10	76 ± 6
MCA 106 sarcoma	3 ± 1	53 ± 1	Allogeneic sarcoma	7 ± 2	88 ± 3
EL-4 lymphoma	−20 ± 2	43 ± 2	Colon cancer	−16 ± 1	62 ± 1
Normal lymphocyte	−12 ± 3	8 ± 2	Esophageal cancer	0 ± 4	78 ± 5
NK target YAC	20 ± 1	72 ± 1	Adrenal cancer	−16 ± 1	68 ± 2
			Pancreatic cancer	−5 ± 1	28 ± 5
			Normal lymphocyte	−9 ± 3	4 ± 1
			NK target K562	46 ± 3	105 ± 6

* Effector:target ratios in the mouse were 100:1 and in humans were 40:1.

(Modified from references 9 and 10).

not been very successful in experimental animal models, it has received extensive clinical testing in a variety of histologic types of cancer.[1] These clinical trials have been disappointing, and immunotherapeutic approaches based on nonspecific stimulation of the body's immune system have, for the most part, been abandoned.

An alternate approach to immunotherapy is that of adoptive immunotherapy, which is defined as the transfer of immunologic reagents with antitumor reactivity to the tumor bearing host.[2-3] Immunologic analyses have shown that it is the cellular arm of the immune system that is largely responsible for tissue rejection, and thus extensive efforts have been made to identify lymphocyte subpopulations with antitumor reactivity. The major obstacle to the application of this approach to cancer treatment has been the inability to raise immune cells with antitumor reactivity in numbers sufficient for use in immunotherapy. Recently, a convergence of the increasing knowledge of cellular immunology and developments in recombinant DNA biotechnology have provided new opportunities for producing such cells.

We have recently described two different techniques for generating cells that serve as suitable candidates for use in the immunotherapy of cancer in humans.[4-6] In 1980, we described the lymphokine activated killer (LAK) cell phenomenon, and more recently, methods for isolating tumor infiltrating lymphocytes (TIL) that are specifically reactive with human tumors. In this report, we will review the experimental basis for these immunotherapy approaches and summarize current clinical applications.

Lymphokine Activated Killer (LAK) Cells

In 1980, we first described a technique for generating lymphoid cells from both mice and humans that were capable of lysing fresh tumor cells, but not normal cells.[4-5] With the incubation of resting murine splenocytes or human peripheral blood lymphocytes in the lymphokine, interleukin-2 (IL-2) results 3 to 4 days later in the generation of cells capable of lysing fresh tumor cells, but not fresh normal cells. These killer cells have been referred to as lymphokine activated killer (LAK) cells.[7-10] Typical experiments illustrating the generation of LAK cells in both mice and humans are shown in Table 1. As this table shows, fresh murine splenocytes or fresh human peripheral blood lymphocytes (PBL) exhibit no lysis against a variety of fresh syngeneic autologous or allogeneic tumor targets. However, the incubation of these resting lymphocytes in IL-2 gives rise to LAK cells that, in the mouse, are capable of killing a variety of syngeneic sarcomas, and in humans, are capable of lysing the autologous cancer as well as a variety of allogeneic cancers.

The characteristics of both murine and human LAK cells have been extensively studied. These cells represent a lytic population quite distinct from natural killer cells and cytolytic T lymphocytes, and their phenotypic surface markers are characteristic of non-MHC restricted killer cells.[8,11] IL-2 represents the sole signal required for the generation of LAK cells, as demonstrated in experiments using purified homogenous recombinant IL-2.[12] Detailed studies in murine models have shown that normal cells from a variety of organs are not lysed by LAK cells and, as demonstrated in cold target inhibition experiments, do not bear cell surface structures recognized by LAK cells.[13] The nature of the determinants recognized by LAK cells on fresh tumor targets is not known and is under intensive study. The natural function of LAK cells is also unknown, although it is tempting to speculate that these cells represent a component of a natural immunosurveillance system against transformed or otherwise altered cells.

Experimental Studies of the Treatment of Established Murine Tumors with IL-2 and LAK Cells

After the description of the LAK cell phenomenon, studies were undertaken to determine whether the adoptive transfer of these cells would be of value in the adoptive immunotherapy of established tumors. Relatively large amounts of IL-2 were required, and the tiny quantities produced by stimulated lymphoid cells presented a severe obstacle to these studies. The isolation of the gene for IL-2, its expression in *Escherichia coli,* and the purification of the resulting IL-2 to homogeneity provided the large amounts required for *in vivo* experiments.[12,14]

A variety of animal models were developed to test the therapeutic efficacy of LAK cells and IL-2.[15-36] Lung metastases were induced in mice by the intravenous injection of tumor cells, and liver metastases were induced by the injection of tumor cells into the exteriorized spleen, allowing cells to flow into the portal circulation and lodge in the liver. After allowing metastases to establish in lung and liver, respectively, experiments were conducted in which animals were treated with LAK cells and IL-2 in various combinations. Table 2 shows typical experiments in which the therapy of murine lung and

TABLE 2. *Treatment of Established Murine Lung and Liver Metastases with LAK Cells and IL-2*

Cells*	Number of Metastases‡			
	Lung Metastases		Liver Metastases	
	No IL-2	Plus IL-2†	No IL-2	Plus IL-2†
None	228	152	246	53
Fresh splenocytes	183	156	245	60
Cultured splenocytes	214	191	250	50
LAK	200	20	193	1

* 10^8 cells administered I.V. on Days 3 and 6 after tumor injection.
† 7500 (liver experiment) and 15,000 (lung experiment) units every 8 hours intraperitoneal Days 3–8 after tumor injection.
‡ Evaluated at 2 weeks after tumor injection. (Modified from references 15 and 18).

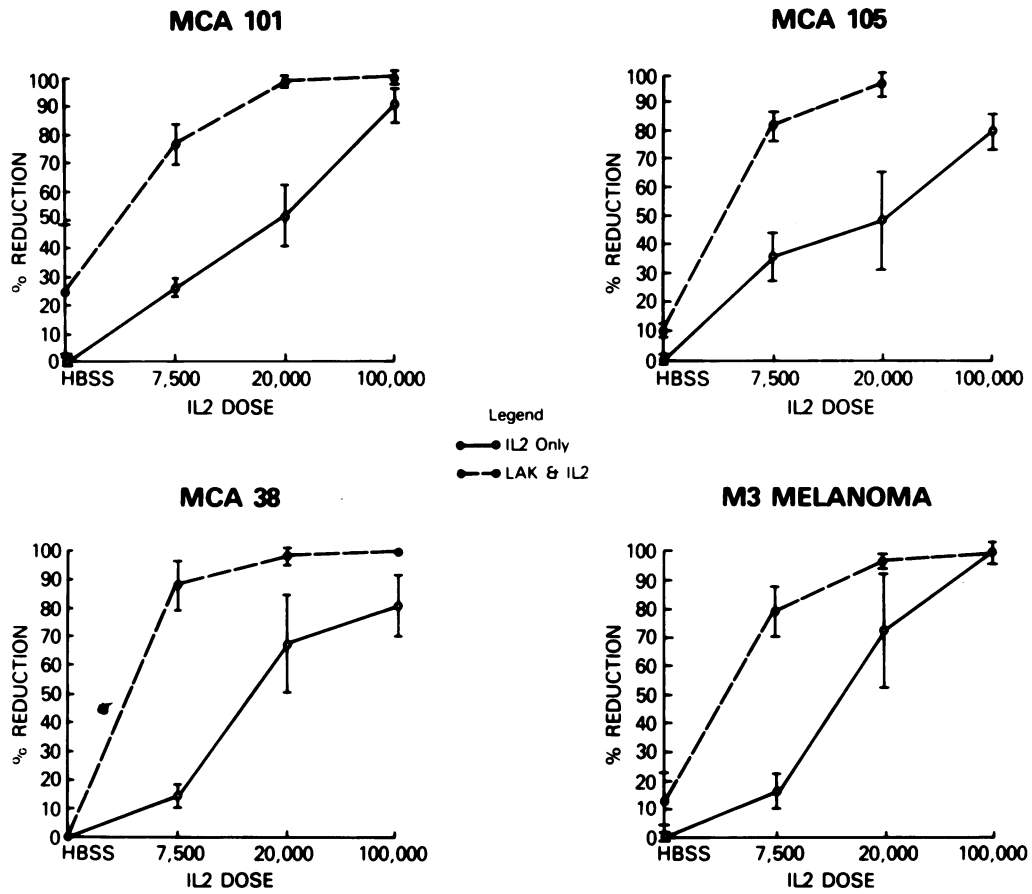


FIG. 1. The treatment of 3-day lung metastases from four different tumors with either high dose IL-2 alone (O) or with high dose IL-2 in conjunction with LAK cells (O). The MCA 101 is a nonimmunogenic sarcoma and the MCA 105 is an immunogenic sarcoma in C57BL/6 mice. The MCA 38 is a murine colon adenocarcinoma in C57BL/6 mice, and the M-3 tumor is a melanoma in C3H mice. High dose IL-2 can mediate antitumor effects when administered alone, although these antitumor effects can be markedly increased by the simultaneous transfer of LAK cells.[75]

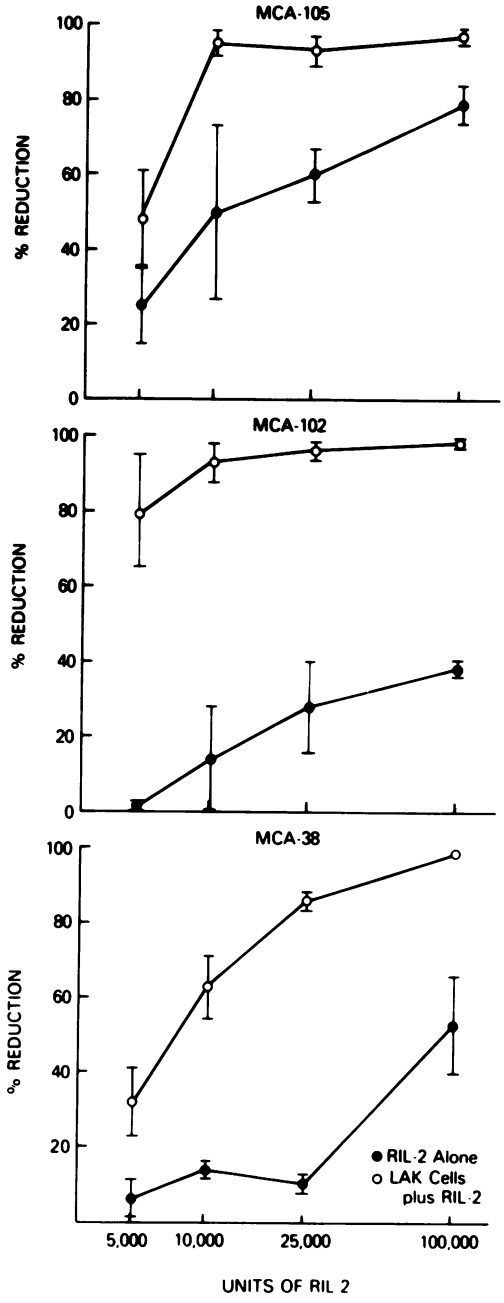


FIG. 2. The treatment of 3-day liver metastases from three different tumors with either high dose IL-2 alone (O) or with high dose IL-2 in conjunction with LAK cells (O). The MCA 102 is a nonimmunogenic sarcoma and the MCA 105 is an immunogenic sarcoma in C57BL/6 mice. The MCA 38 is a murine colon adenocarcinoma. High dose IL-2 can mediate antitumor effects when administered alone, although these antitumor effects can be markedly increased by the simultaneous transfer of LAK cells.[19]

liver metastases from a syngeneic sarcoma were studied. Animals receiving therapy with fresh splenocytes, splenocytes cultured in media alone, and splenocytes cultured in IL-2 to produce LAK cells resulted in little therapeutic benefit. Depending on the dose given, the administration of IL-2 alone could reduce metastatic deposits. In the experiment shown in Table 2, the IL-2 dose had little impact on lung metastases, but was capable of significantly reducing the number of liver metastases. When LAK cells were transferred in conjunction with IL-2, an optimal therapeutic benefit was obtained,

TABLE 3. *Immunotherapy of Murine Tumors with IL-2 Alone*

1. Liver and lung micrometastases (3-day) from a variety of immunogenic and nonimmunogenic sarcomas, melanomas, and adenocarcinomas can be inhibited by IL-2 administration.
2. Lung macrometastases (10-day) from two immunogenic sarcomas (but not from two nonimmunogenic sarcomas) can be inhibited by IL-2 administration.
3. A direct relationship exists between the dosage of IL-2 and therapeutic effect.
4. High dose IL-2 administration leads to *in vivo* lymphoid proliferation in visceral organs. These cells have LAK activity *in vitro*.
5. The immunotherapeutic effect of IL-2 on 3-day micrometastases is mediated by asialo GM1-positive LAK cells. In immunogenic tumors, Lyt 2-positive cells also participate.
6. The immunotherapeutic effect of IL-2 on 10-day macrometastases in mediated by Lyt 2-positive cells.
7. Immunosuppression with irradiation or cyclophosphamide can inhibit IL-2 therapy against 3-day metastases, but can enhance the effects of IL-2 on 10-day macrometastases.
8. The sensitivity of macrometastases to therapy involving IL—2 appears to be directly related to the expression of MHC antigens (Class I) on the tumor.
9. The administration of IL-2 can enhance the therapeutic effect of concomitantly administered LAK cells, TIL, and specifically sensitized T lymphocytes.

TABLE 4. *Immunotherapy of Murine Tumors with LAK Cells Plus IL-2*

1. Liver and lung micrometastases (3-day) from a variety of immunogenic and nonimmunogenic sarcomas, melanomas, and adenocarcinomas can be inhibited by treatment with LAK cells plus IL-2.
2. A direct relationship exists between therapeutic effect and the dosage of IL-2 and the dose of LAK cells.
3. The precursor of the LAK cell effective *in vivo* is $Thy1^-$ Ig^-Ia^-ASGM1.
4. Three-day incubation of splenocytes is optimal for the generation of LAK cells effective *in vivo*.
5. Immunotherapy of micrometastases with LAK cells and IL-2 is effective in hosts suppressed by total body irradiation or treatment with cyclophosphamide. Therapy is also effective in "B" mice (thymectomized, lethally irradiated, reconstituted with T cell depleted bone marrow).
6. Immunotherapy of micrometastases with allogeneic LAK cells plus IL-2 is effective.
7. LAK cells effective in immunotherapy can be generated from the splenocytes of tumor-bearing mice.
8. Metastases that persist after *in vivo* therapy with LAK cells plus IL-2 are sensitive to LAK cell lysis both *in vitro* and in subsequent *in vivo* experiments. We have been unable to generate LAK-resistant tumor cells.
9. Administration of IL-2 leads to *in vivo* proliferation of transferred LAK cells.
10. Diffuse intraperitoneal carcinomatosis ca be successfully treated with intraperitoneal LAK cells plus IL-2.
11. LAK cells can mediate antibody dependent cellular cytotoxicity, and this administration of IL-2 alone or LAK cells plus IL-2 can enhance the *in vivo* therapeutic efficacy of monoclonal antibodies with antitumor reactivity.

TABLE 5. *Surgery Branch, NCI Clinical Studies Using IL-2 for the Treatment of Cancer*

Year	Clinical Study	No. of Patients	Reference	Findings
1980	Adoptive transfer of long-term cultured peripheral blood lymphocytes	3	37	Small numbers (up to 5×10^8) of long-term cultured peripheral blood lymphocytes could be safely infused in humans
1981	Adoptive transfer of phytohemagglutinin activated killer (PAK) cells	10	80	Large numbers (up to 1.7×10^{11}) of activated killer cells, obtained from up to 15 successive leukaphoreses, could be infused safely in humans
1982	Adoptive transfer of PAK cells plus cyclophosphamide	6	81	Activated killer cells could be safely infused in conjunction with high dose cyclophosphamide (50 mg/kg)
1983	Adoptive transfer of PAK cells plus activated macrophages	5	81	Activated killer cells plus activated macrophages could be safely infused
1983	Administration of natural (Jurkat-derived) IL-2	16	48	Natural (Jurkat-derived) IL-2 could be safely infused into humans at doses of up to 2 mg
1984	Adoptive transfer of lymphokine-activated killer LAK cells	6	81	LAK cells (activated with recombinant IL-2) could be safely infused in humans
1984	Administration of recombinant IL-2	23	49	Recombinant IL-2 (from E. coli) could be safely administered though significant toxicity at high doses
1985	Administration of LAK cells plus recombinant IL-2	25	15	Regression of metastatic cancer of a variety of types in some patients
1986	Administration of high dose bolus IL-2 alone	10	16	Regression of metastatic cancer in three patients with melanoma
1987	Administration of IL-2 alone or with LAK cells	157	17	Complete and partial regression of cancer of several histologic types
1988	Administration of IL-2 alone or with LAK cells	222	Current report	Complete and partial regression of cancer of several histologic types

with a 90% reduction in the number of established lung metastases and greater than 99% reduction in the number of established liver metastases.

Extensive dose response studies were performed using therapy with both IL-2 alone, as well as with IL-2 in conjunction with LAK cells. Figure 1 shows the results of multiple experiments in which the treatment of pulmonary metastases from four different tumors was studied; Figure 2 shows the results of experiments dealing with the treatment of liver metastases from three different tumors. Several important points are illustrated in these experiments. At increasing doses of IL-2 alone, significant therapeutic effects were seen in the treatment of lung and liver metastases. At the highest doses of IL-2, the results were often comparable to those of the transfer of LAK cells plus IL-2. However, with most doses of IL-2, the concomitant transfer of LAK cells improved the efficacy of treatment. Therapy for Day 3 metastases with IL-2 plus LAK cells appeared effective against nonimmunogenic sarcomas, such as the MCA-101 and MCA-102 tumors, as well as against the immunogenic sarcoma, MCA-105. There was likewise therapeutic benefit in the cases of the murine colon adenocarcinoma 38 and the M3 melanoma.

Tables 3 and 4 present a summary of the results of studies of the immunotherapy of murine tumors, using either IL-2 alone or LAK cells plus IL-2.[15-36] These studies laid the groundwork for the application of treatment using IL-2 alone or IL-2 plus LAK cells in humans.

Clinical Studies of the Immunotherapy of Cancer in Humans Using IL-2 and LAK Cells

Table 5 presents a chronologic summary of the clinical immunotherapy studies performed at the Surgery Branch of the National Cancer Institute (NCI).[37-44]

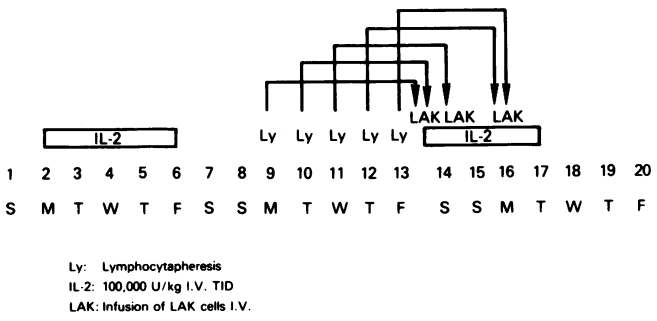


FIG. 3. Clinical protocol for the immunotherapy of human cancer with LAK cells plus recombinant IL-2. IL-2 is administered for 4–5 days, resulting in a marked lymphocytosis that increases the yield of lymphocytes obtained from daily lymphocytophereses. Lymphocytes are cultured to produce LAK cells and are then reinfused in patients simultaneously with the administration of IL-2.

TABLE 6. *Results of Immunotherapy in Patients with Advanced Cancer*

	LAK/IL-2			IL-2		
		Number of Patients			Number of Patients	
Diagnosis	Total Evaluable*	CR	PR	Total Evaluable†	CR	PR
Renal	54	7	10	38	4	3
Melanoma	34	3	3	23	0	6
Colorectal	27	1	2	10	0	0
Non-Hodgkin's Lymphoma	4	1	2	3	0	0
Sarcoma	6	0	0	1	0	0
Lung adenocarcinoma	5	0	0	1	0	0
Breast	2	0	0	1	0	0
Brain	1	0	0	2	0	0
Esophageal	1	0	0	0	0	0
Hodgkin's Lymphoma	1	0	0	0	0	0
Ovarian	1	0	0	0	0	0
Testicular	1	0	0	0	0	0
Gastrinoma	1	0	0	0	0	0
Unknown primary	1	0	0	0	0	0
Total	137	12	17	80	4	9

* Two treated patients not included died of therapy (melanoma) and at follow-up (breast), respectively.

† Three treated patients not included died of therapy (renal).

CR = complete regression.
PR = partial regression.

Shortly after the description of the LAK cell phenomenon in 1980,[5,6] we began preliminary attempts to apply the use of IL-2 and LAK cells in the treatment of cancer in humans. Recombinant IL-2 did not become available until 1984, and when we began our exploratory clinical trials in 1981, sufficient IL-2 was not available for human studies. Other techniques for generating large numbers of activated killer cells capable of killing fresh human tumors were identified, such as the incubation of resting human lymphocytes with lectin (e.g., phytohemagglutinin and concanavalin A) and the use of pooled allogeneic mixed lymphocyte interactions.[45-50] These *in vitro* stimulations appeared to result in activated killer cells by inducing the secretion of IL-2 into the culture medium. In 1981, therefore, we began clinical trials with phytohemagglutinin activated killer (PAK), and showed that large numbers of these cells (up to 1.7×10^{11}) obtained from successive leukaphereses could be safely administered to patients.[38] Ten patients received the adoptive transfer of PAK cells alone, six received PAK cells plus cyclophosphamide, and five received PAK cells plus activated macrophages.[38,39] These and subsequent trials were conducted in patients who either had advanced cancer that had failed all standard treatments, or who had malignancies for which there was no standard effective treatment available. When recombinant IL-2 became available, six patients were treated with the

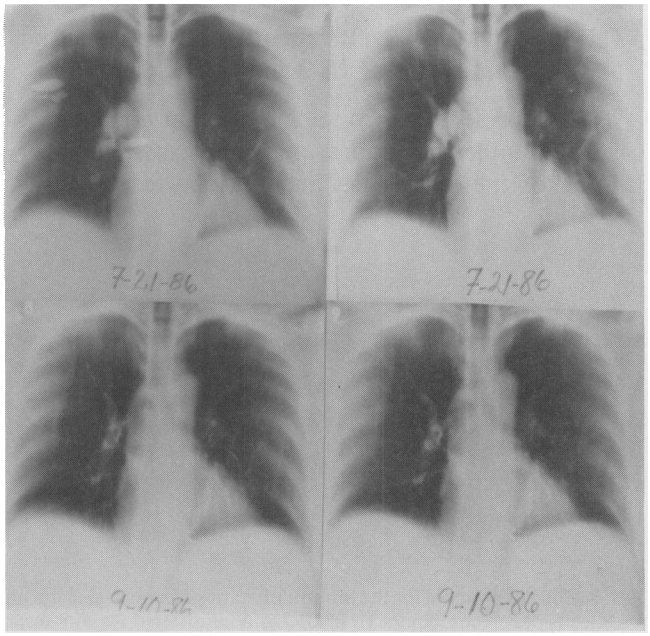


FIG. 4. Complete regression of a hilar metastasis from melanoma in a patient treated with LAK cells and IL-2: Pretreatment (top) and post-treatment (bottom).

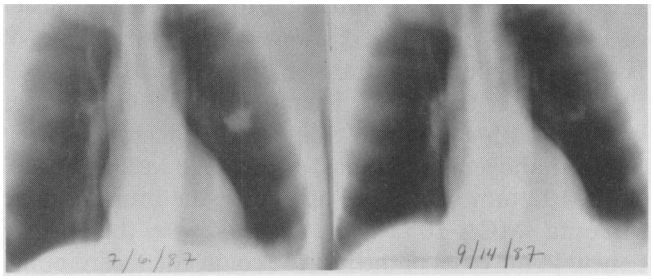
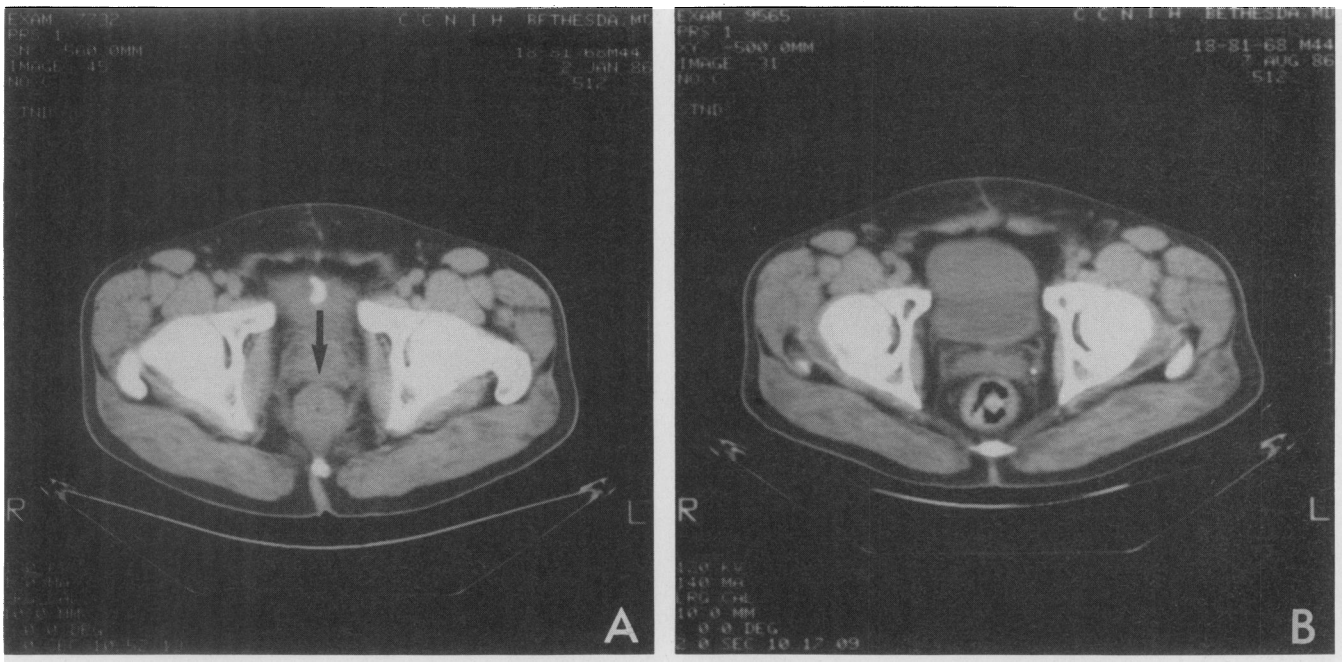


FIG. 5. Partial regression of pulmonary metastases in a patient with renal cell cancer treated with LAK cells and IL-2: Pretreatment (left) and post-treatment (right).



FIGS. 6A and B. Complete regression of a recurrent tumor mass at the site of a low anterior resection for colorectal cancer in a patient treated with LAK cells and IL-2: (A) Pretreatment (arrow points to the mass at the site of the anastomosis) and (B) post-treatment. This patient also underwent complete regression of lung and liver metastases.

adoptive transfer of LAK cells produced using recombinant IL-2.[39] No antitumor responses were seen in any of these 27 patients treated with activated killer cells alone.

In 1983, we began a clinical trial in patients with advanced cancer using the administration of natural IL-2 derived from a high-producing human cell line.[40] Thirty milligrams of pure IL-2 were isolated from hundreds of thousands of liters of culture supernatant, and this purified material was then infused into humans. When recombinant IL-2 became available, we administered much higher doses of this purified material to cancer patients through a variety of routes, including intravenous (I.V.) bolus injection, continuous I.V. infusion, intratumor injection, and intraperitoneal injection.[41] Our studies established the dose-limiting toxicity of IL-2 and identified significant toxicities associated with its administration. In these Phase I studies, 39 patients were treated with either the natural or recombinant IL-2, and none of the patients exhibited any antitumor effects.

In November 1984, we received permission from the Food and Drug Administration to begin clinical trials in advanced cancer patients, utilizing a combination of LAK cells plus recombinant IL-2.[42] Although this protocol changed in minor ways as our experience developed, an outline of the basic protocol used in most patients is given in Figure 3. A cycle of IL-2 alone was given that was followed by a profound peripheral lymphocytosis. Five daily leukaphereses were then performed, and these resulting lymphocytes were cultured to produce LAK cells. As had been required for optimal therapeutic effect in our animal models, the LAK cells were then reinfused simultaneously with the administration of IL-2. With this combined treatment, metastatic cancer of a variety of types regressed in selected patients.[42] As experience accumulated with the administration of high-dose IL-2, clinical trials were instituted using high dose IL-2 alone, and, as was predicted in our animal models, regression of metastatic cancer was seen with this treatment, as well.[43] The doses of IL-2 used in these latter trials were higher than those used in our earlier, Phase I efforts. In 1987, we reported the results of 157 patients who were administered high dose IL-2 alone or with LAK cells;[44] in this review this information is updated to include the treatment of 222 patients with advanced cancer accrued by 5/1/87 and assessed as of 7/1/87. Eighty-three patients were treated with high

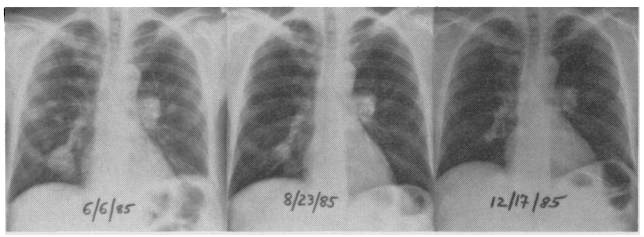
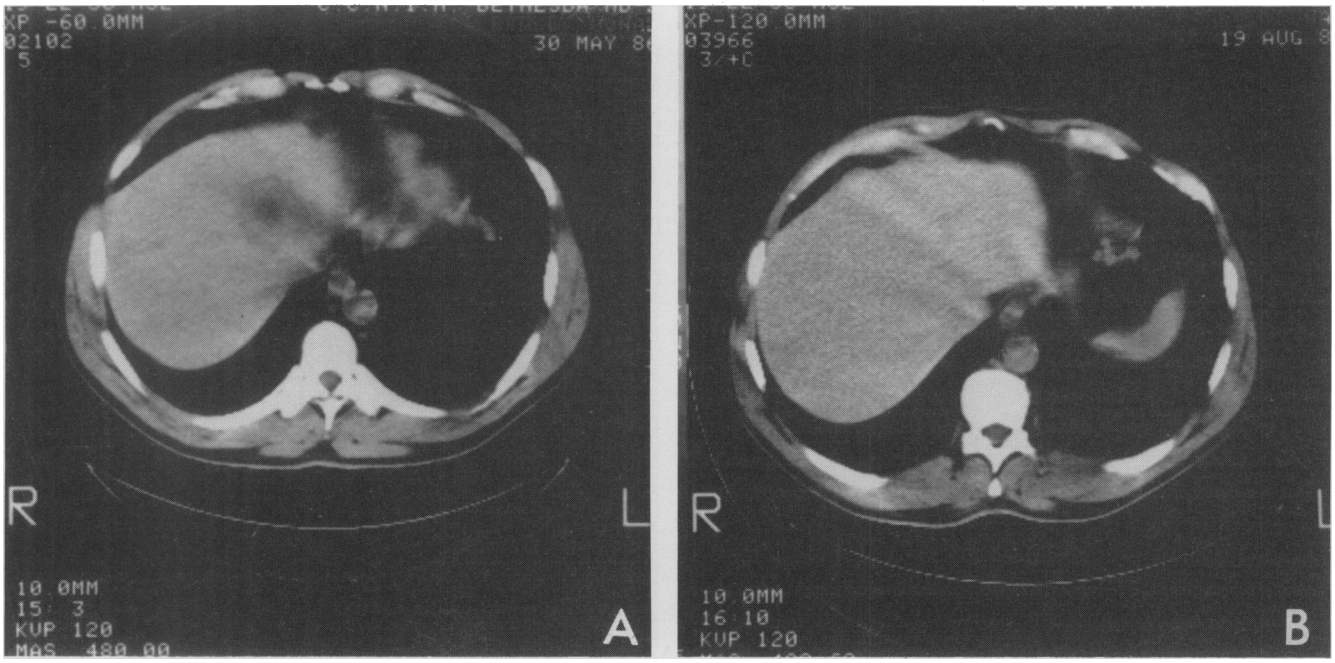


FIG. 7. Partial regression of pulmonary metastases in a patient with renal cell cancer treated with LAK cells and IL-2: Pretreatment (left) and post-treatment (middle and right).



FIGS. 8A and B. Regression of a liver metastasis in a patient with melanoma treated with high dose IL-2: (A) Pretreatment and (B) post-treatment.

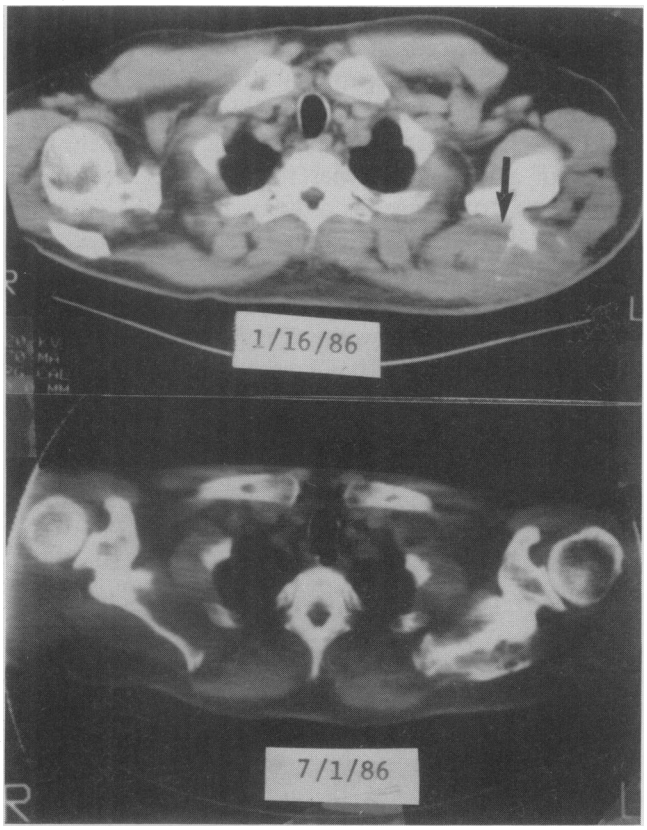


FIG. 9. Complete regression of a scapular metastasis in a patient with renal cell cancer treated with LAK cells and IL-2. (top) pretreatment (arrow points to the tumor in the scapula that caused a large soft tissue mass). (bottom) regression of the tumor with remineralization of the scapula.

dose IL-2 alone, and 139 were treated with LAK cells plus IL-2. The results of the treatments are shown in Table 6.

Sixteen complete responses and 26 partial responses (defined as greater than 50% reduction of the sum of the perpendicular diameters of all lesions) were seen. Additional patients experienced 25–50% reductions in tumor. Regressions of metastatic cancer were seen in patients with renal cell cancer, malignant melanoma, colorectal cancer, and non-Hodgkin's lymphoma. Very few patients with other histologic types of cancer have been treated. It appears that some tumors are more responsive than others, although additional experience is required to confirm this observation. Regression of metastatic cancer has been seen at a variety of sites, including lung, liver, bone, skin, subcutaneous tissue, and in circulating tumor cells. When tumor regression occurred at one site, it tended to occur at *all* sites; mixed responses were unusual. Figures 4–14 show examples of antitumor responses in selected patients.

The mechanism of the antitumor effect appeared to be similar to that demonstrated in the mouse. IL-2 probably results in the generation of LAK cells *in vivo,* however, it is also capable of simulating endogeneous antitumor responses. In several patients who have undergone biopsies during treatment, extensive infiltrates of lymphocytes in the tumor have been observed (Fig. 15).

The duration of responses is shown in Table 7. Of the 16 patients who underwent complete regression, 13 remained in complete regression from 2 to 31 months.

The median duration of complete responses was not reached, and the median duration of partial responses was 6 months.

Meaningful clinical responses have been seen in patients receiving high dose IL-2 alone as well as in patients receiving LAK cells plus high dose IL-2. It is not clear whether the adoptive transfer of LAK cells is necessary in all clinical situations where very high doses of IL-2 are used. The Surgery Branch of the NCI is now conducting a trial in which patients with advanced cancer are randomized to receive either high dose IL-2 alone or high dose IL-2 in conjunction with LAK cells.

Toxicity of Treatment

In early studies, administration of activated killer cells alone caused little toxicity.[38,39] However, administration of high dose recombinant IL-2 was associated with substantial dose-limiting toxicity in a variety of organ systems.[44,51-55] The major side effects of IL-2 can probably be attributed to three major factors. IL-2 causes a lymphoid infiltrate that can be quite marked and can interfere with the function of vital organs. In addition, IL-2 induces a vascular permeability leak that leads to fluid retention and interstitial edema, which likewise can compromise organ function.[56,57] Finally, IL-2 most likely leads to the secretion of other lymphokines, with a myriad of physiologic effects. When IL-2 administration ceases, all side effects appear to be completely reversible.

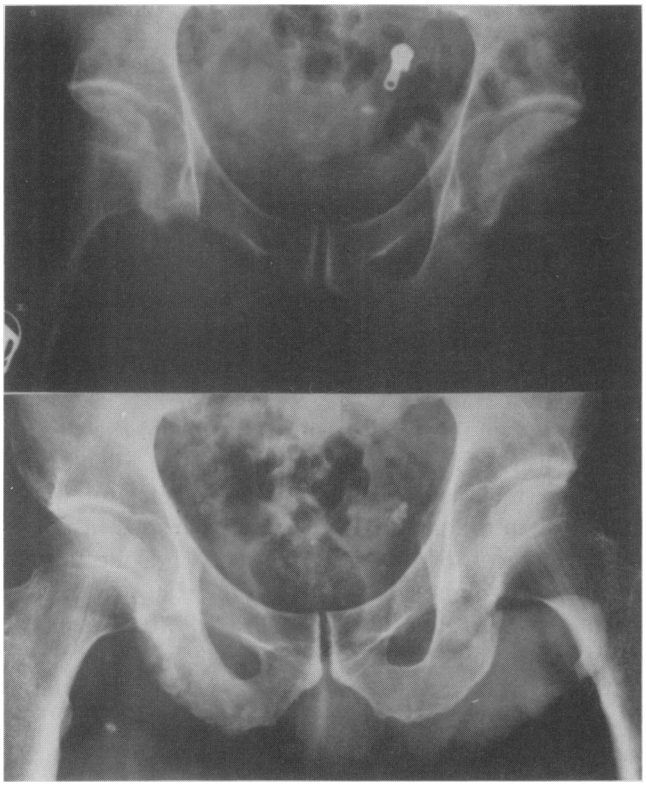


FIG. 10. Complete regression of a boney metastasis of the pubic ramus in a patient with renal cell cancer treated with LAK cells and IL-2. Pretreatment (top) and post-treatment (bottom). This patient also underwent complete regression of multiple pulmonary metastases.

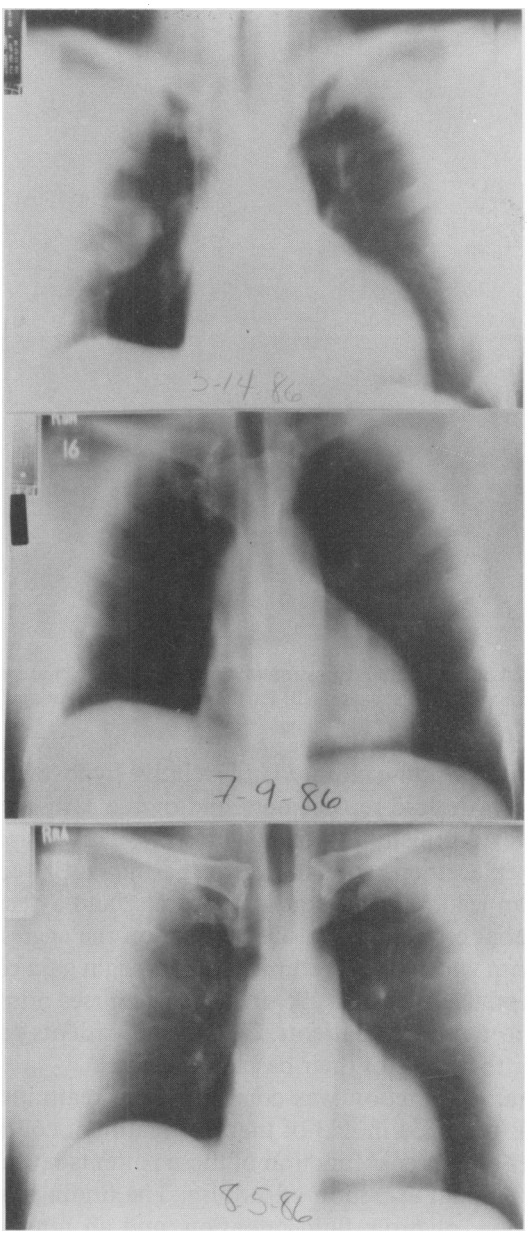
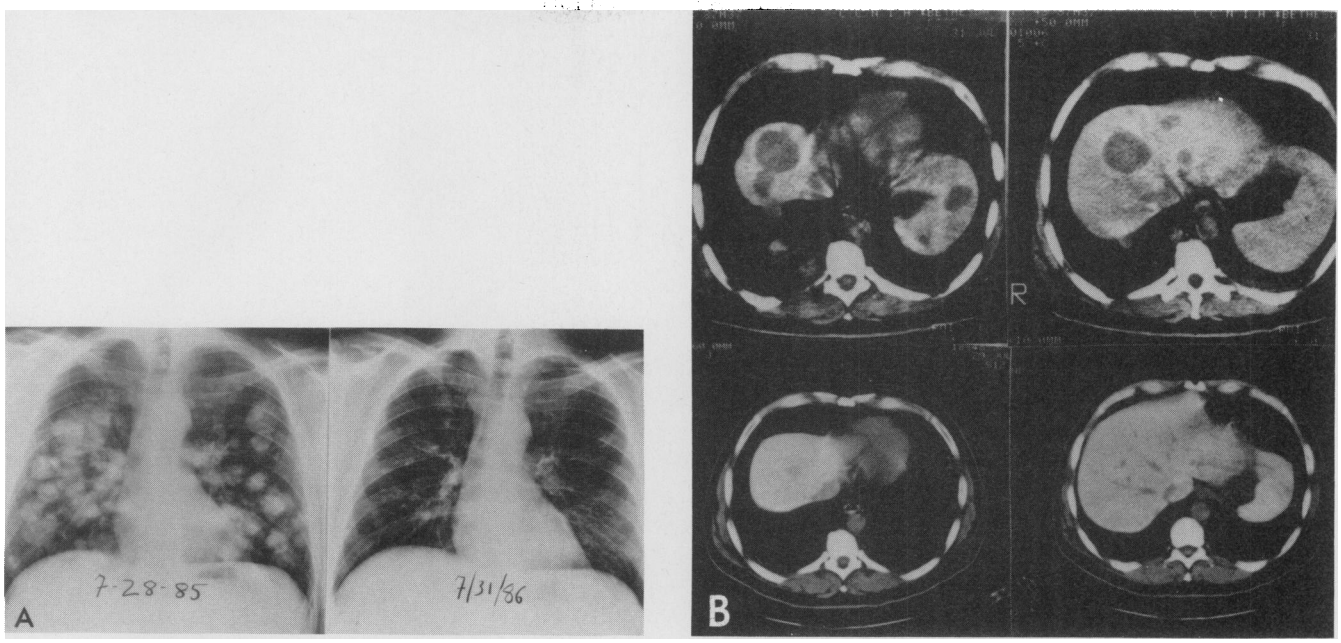


FIG. 11. Complete regression of pulmonary metastasis in a patient with renal cell cancer treated with high dose IL-2 alone. Pretreatment (top) and post-treatment (middle and lower).

Figure 16 illustrates the clinical course and many of the physiologic problems of patients receiving IL-2 plus LAK.[55] Shortly after the administration of IL-2, there is a profound drop in systemic vascular resistance, with a concomitant tachycardia, a decrease in mean arterial blood pressure, and an increase in cardiac index. As IL-2 administration continues, weight gain increases and urine output drops. In an attempt to decrease the



FIGS. 12A and B. Partial regression of lung, liver, and splenic metastases in a patient with melanoma treated with high dose IL-2 alone. (A) Partial regression of lung metastases. (B) Partial regression of liver and splenic metastases.

amount of volume required, this relative hypovolemia is treated with fluid replacement and by the early and aggressive administration of vasopressors. This obligatory fluid replacement contributes to the interstitial edema that can lead to respiratory compromise and a decrease in arterial oxygenization. Depending on the aggressiveness with which fluid is administered, weight gain can be substantial; in 188 of 295 treatment courses administered to these 222 patients, 27% of the patients gained greater than 10% of their baseline weight.

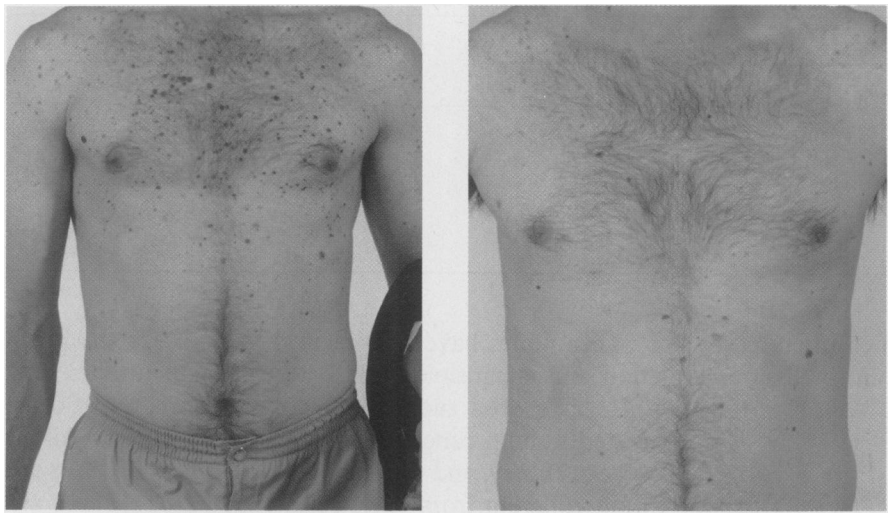
Renal dysfunction was common and creatinine elevations occurred in 243 of the 295 treatment courses.[51] Studies of the renal function of these patients revealed a low fractional excretion of sodium. The findings of hypotension, oliguria, fluid retention, and an intense tubular avidity for sodium support a prerenal mechanism for the renal dysfunction based on reduced renal perfusion.[51] As with virtually all of the other side effects of IL-2, these changes are transient, and all patients returned to baseline creatinine levels, with a median time of returning to normal of 4 days. Other toxicities in these patients included hematologic abnormalities that often resulted in anemia and thrombocytopenia, nausea, vomiting, hyperbilirubinemia, and confusion.[52–54] These side effects promptly reverse after IL-2 administration. The median treatment course lasted 16 days, and the median time from the end of treatment to discharge from the hospital was 5 days.

Several other groups have now confirmed the antitumor effects of treatment with LAK cells and IL-2.[58–62] It has been suggested that the continuous infusion of IL-2 causes less toxicity than bolus administration;[58] however, direct comparison between these two methods of IL-2 administration have not been performed. The concomitant administration of steroids can also lead to decreased toxicity of IL-2 administration. Nevertheless, experimental studies indicate that this may abrogate therapeutic benefit as well.[63,64]

Newer Approaches to Immunotherapy

Intensive efforts are underway to identify cells with greater effectiveness for use in adoptive transfer. In 1980, we described a method for isolating pure cultures of lymphocytes infiltrating into solid tumors.[4] These tumor-infiltrating lymphocytes (TIL) have interesting properties.[65–67] We have recently shown that TIL have far greater therapeutic potency than LAK cells in experimental animal models.[6,68]

TIL are lymphoid cells that infiltrate into the stroma of established growing tumors and can be isolated by culturing single cell suspensions from resected tumors in high concentrations of IL-2. The IL-2 results in the *in vitro* expansion of activated T cells within the tumor, and as the lymphocytes expand, they result in the destruction of surrounding tumor cells. After 2–3 weeks of culturing, pure populations of lymphoid cells devoid of tumor cells are present. When these TIL populations were studied in experimental tumor models, they had far greater effectiveness than did LAK cells.[6,68] A comparison of the relative efficacy of LAK cells and TIL is shown in Figure 17. Whereas 2×10^8 LAK cells were required to mediate complete elimination of established 3-day pulmonary metastases, 2×10^6 TIL accomplished this same therapeutic effect. After these studies, we



FIGS. 13. Complete regression of multiple cutaneous metastases in a patient with malignant melanoma treated with LAK cells and IL-2. Pretreatment (left) and post-treatment (right).

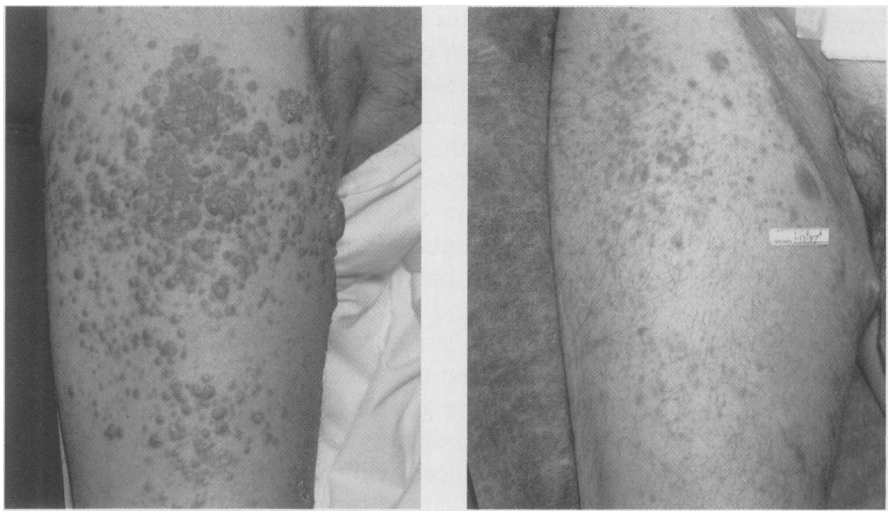


FIG. 14. Partial regression of multiple cutaneous metastases in the thigh of a patient with melanoma treated with high dose IL-2 alone. Pretreatment (left) and post-treatment (right).

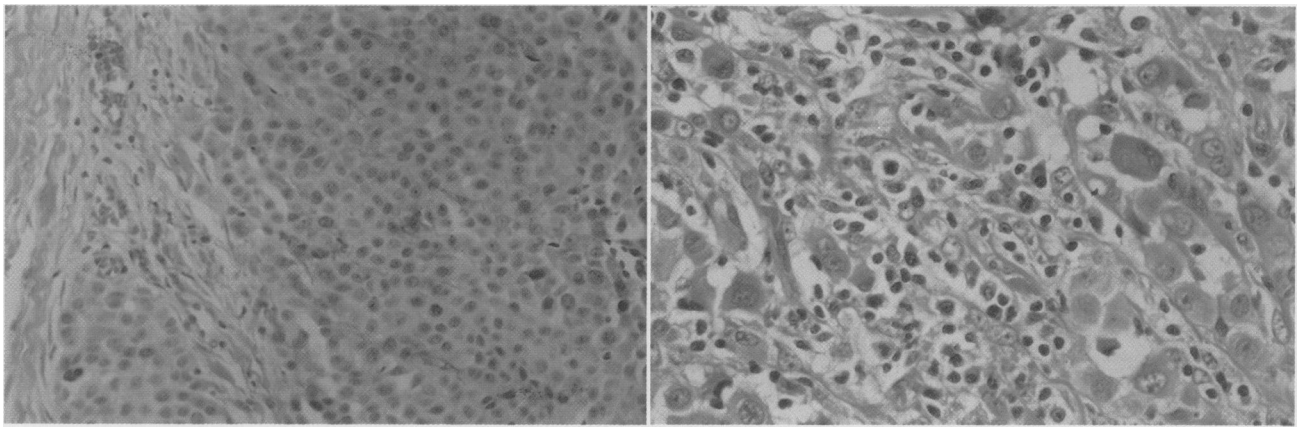


FIG. 15. Pre- and post-treatment biopsies of a patient with subcutaneous nodules from malignant melanoma treated with LAK cells and IL-2. Pretreatment (left) and post-treatment (right). After treatment with LAK cells and IL-2, the architecture of the subcutaneous tumor nodules was disrupted, and an infiltrate of activated lymphocytes appeared in the tumor.

tested the use of these TIL in advanced tumor models in which IL-2 alone or IL-2 plus LAK cells had no therapeutic benefit. As was the case with the adoptive transfer of T cells in other animal models, some form of immunosuppression was necessary to mediate therapeutic effects. In these studies, a combination of cyclophospha-

TABLE 7. *Duration of Responses*

Diagnosis	LAK/IL-2 (months)		IL-2 (months)	
	CR	PR	CR	PR
Renal	14+, 12+, 9+, 9 7+, 6, 2+	15+, 11+, 6+, 6 6, 6, 6, 2, 1, 1	13+, 7+, 6+ 4+	7+, 7, 3
Melanoma	31+, 11+, 11+	6, 2, 2	—	20, 12+, 10, 8, 7, 2
Non-Hodgkins Lymphoma	10	11+, 7+	—	—
Colorectal	17+	6, 2	—	—

mide plus TIL and IL-2 were capable of eliminating the large tumor burdens that were present in the liver and lungs of mice.[6]

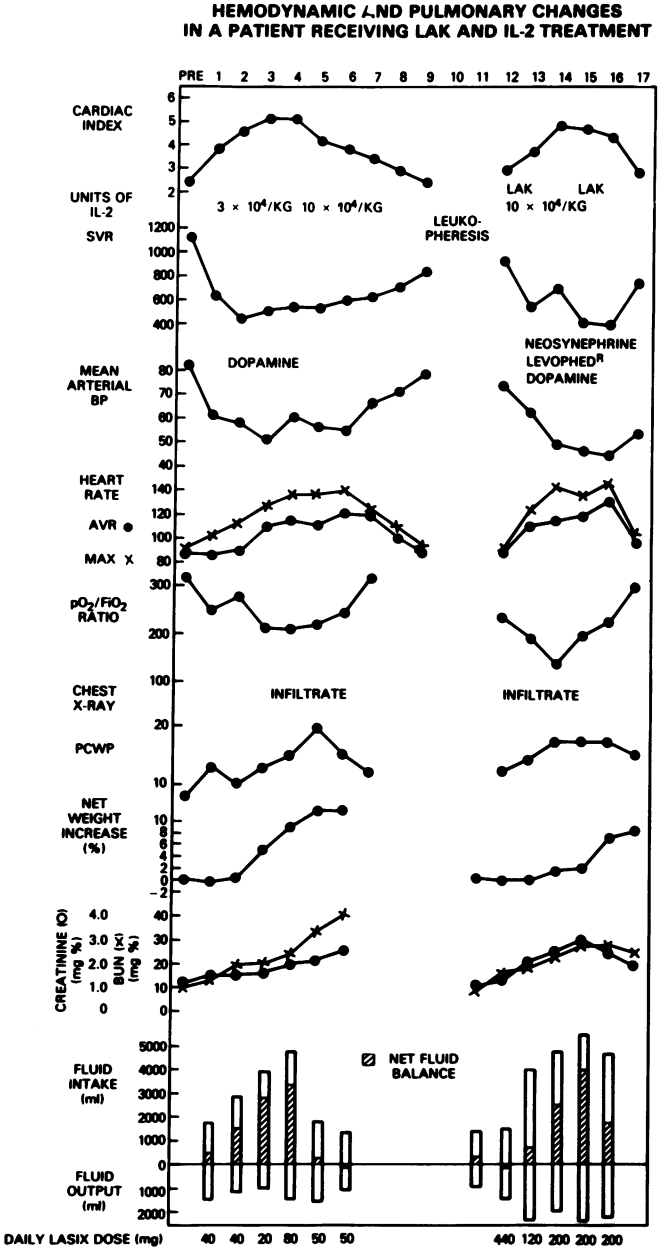


FIG. 16. Sequential clinical measurements of a patient receiving treatment with LAK cells and IL-2.[55]

Techniques have now been developed for the isolation and expansion of TIL from human tumors, and TIL have been successfully grown from over 100 resected human cancers, including cancers of the colon, breast, kidney, and melanoma.[69–72] Human TIL cells are quite different from LAK cells. Whereas LAK cells are derived from non-B, non-T lymphocytes, TIL are derived from T cells and when expanded in culture they have the phenotype of classic cytolytic T lymphocytes.[69]

From approximately half of patients with malignant melanoma, TILs can be isolated with unique cytolytic specificity for the tumor from which they were derived.[69] By contrast, LAK cells are broadly specific in their lysis. Figure 18 shows the lytic specificity of TIL from three patients with melanoma. These TIL are capable of lysing only the melanoma from which they were derived, and not the other two allogeneic melanomas. TIL are also specific for tumor and do not lyse normal cells from the same patient.

Because of the greater potency of TIL as compared with LAK and the ability to isolate specific cytolytic TIL from human cancers, clinical trials of the use of TIL in patients with advanced cancer are currently underway.

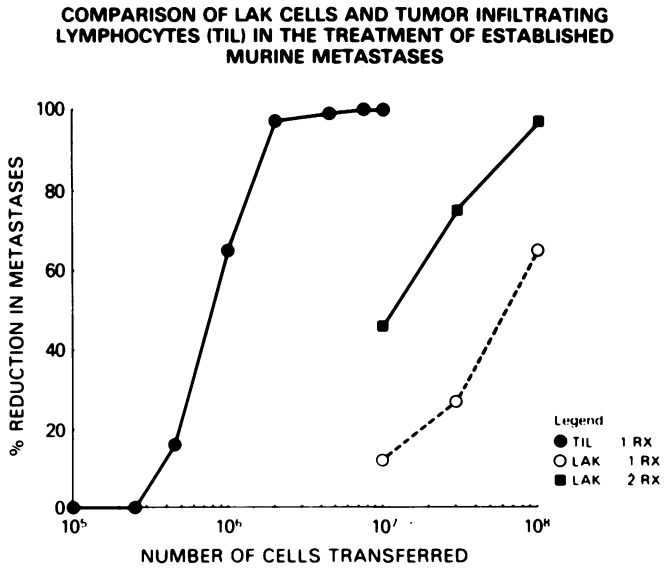


FIG. 17. Titration of the number of LAK cells and TIL required to reduce established 3-day pulmonary metastases in a murine tumor model.[68] On a per cell basis, TIL appear to be 50–100 times more potent than LAK cells.

Methods are also being developed to enhance the immunotherapeutic efficacy of therapy with IL-2. Recent experiments in animal models have shown that other lymphokines, such as tumor necrosis factor and alpha interferon, provide synergistic therapeutic benefits when given with IL-2. Clinical trials have begun in the Surgery Branch of the NCI, for the treatment of patients with advanced cancer using IL-2 in conjunction with either tumor necrosis factor or alpha interferon. A therapeutic effect has also been seen when using IL-2 combined with chemotherapy,[73] and a clinical trial of combination chemotherapy and immunotherapy has also begun. LAK cells mediate potent antibody dependent cellular cytotoxicity, and thus the combination of therapy with monoclonal antibodies and IL-2 is also being explored.[36,74] The clinical immunotherapy studies now in progress in the Surgery Branch of the NCI are listed in Table 8.

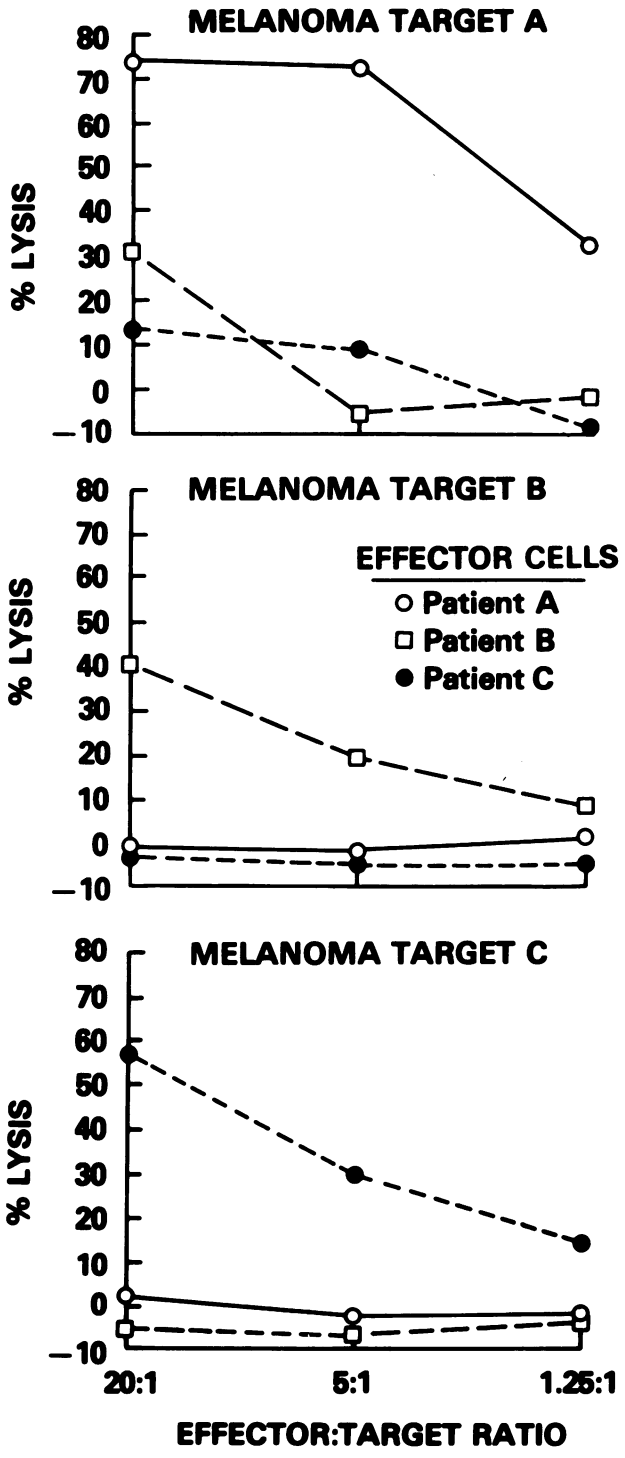


FIG. 18. Lytic specificity of TIL from three patients with melanoma tested simultaneously against fresh melanoma cells. Note that TIL exhibit strong preferential lysis for the target from which they were derived.[69]

TABLE 8. *Clinical Immunotherapy Trials in Progress in the Surgery Branch, National Cancer Institute*

1. Prospective randomized trials
   - Advanced cancer
     - IL-2 alone
     - IL-2 + LAK cells
   - Adjuvant (after lymph node dissection in patients with Stage II melanoma)
     - Surgery (lymph node dissection)
     - Surgery + IL-2
     - Surgery + IL-2 LAK cells
   - Adjuvant (after complete resection of liver metastases from colorectal cancer)
     - Surgery
     - Surgery + IL-2 + LAK cells
   - Adjuvant (after complete resection of locally advanced renal cell cancer)
     - Surgery
     - Surgery + IL-2 + LAK cells
2. Monoclonal antibody plus IL-2 in patients with advanced colorectal cancer
3. Chemotherapy (5 FU + leucovorin) plus IL-2 in patients with advanced breast and colorectal cancer
4. Tumor infiltrating lymphocytes plus IL-2 (plus cyclophosphamide) in patients with advanced cancer
5. Evaluation of new cytokines and combinations of cytokines
   - Tumor necrosis factor (TNF) plus IL-2
   - Alpha-interferon plus IL-2
   - Granulocyte-macrophage colony stimulating factor (GM-CSF) alone or with cyclophosphamide

The studies reported here demonstrate that immunologic manipulations are capable of mediating the regression of established growing cancers in humans. The use of immunotherapy with high dose IL-2 alone or with LAK cells represents a promising approach to cancer treatment. Much effort is currently in progress to increase the efficacy of treatment and decrease toxicity and the complexity of treatment administration. Immunotherapy as a treatment for cancer in humans is still in the infancy of its development, but current efforts appear to represent a base upon which significant improvements in cancer treatment can be made.

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